GENSET.077AUS PATENT

INTEGRATION OF BIOCHEMICAL PROTOCOLS IN A CONTINUOUS FLOW MICROFLUIDIC DEVICE

RELATED APPLICATIONS

[0001] The present application claims priority to French patent application serial No. 99/09806, filed 28 July 1999; French patent application serial No. 99/11652 filed 17 September, 1999; and French patent application serial No. 99/12317 filed October 1, 1999 the disclosures of all of which are incorporated herein by reference in their entireties.

BACKGROUND

[0002] Microfluidics consist of using microchannels instead of test tubes or microplates to carry out analyses and reactions. These microchannels or microcircuits are etched into silicon, quartz, glass, ceramics or plastic. The size of these channels is on the order of micrometers, while the reaction volumes are on the order of nanoliters or microliters. The principle of a microfluidic device is to guide reaction media containing reagents and samples, over zones which correspond to the different steps of the protocol. The integration of reactors, chromatographic columns, capillary electrophoresis systems and miniature detection systems into these microfluidic systems allows the automation of complex protocols by integrating them into a single system. These "laboratories on chips" have made it possible to obtain results which are efficient in terms of reaction speed, in terms of product economy and in terms of miniaturization which allows the development of portable devices. Complex protocols have been integrated and automated, including biochemical or molecular biology protocols which often require extensive manipulation. These manipulations include mixing reagents and samples, controlling the reaction temperature, carrying out thermal cycling, separation by electrophoresis, and detection of reaction products.

[0003] Wolley *et al.* (*Anal. Chem.* 68: 4081-4086 (1996), the contents of which is incorporated herein by reference in its entirety) discloses the integration of a PCR microreactor, a capillary electrophoresis system and a detector in a single device. The PCR reaction, separation of PCR products by electrophoresis, and detection of PCR products are

[0009] Combining the microfluidic substrate with a thermal support makes it possible to control the reaction temperature in the different zones of the channel corresponding to the various steps of the protocol. The invention relates to advantageous devices and processes for carrying out thermal cycling in continuous flow on thermal cycling zones.

[0010] The device is based on a system for distributing and moving the fluids by hydrostatic pressure. All steps of a protocol are carried out in continuous flow; wherein sequential injections of samples and of reagents make it possible to carry out a large number of reactions one after the other in the same channel. Reagents can be injected successively at different stages of the protocol. By arranging several channels in parallel, it is possible to carry out the same protocol in series in the same channel and in parallel in various channels. Synchronizing the reactions in the channels arranged in parallel makes it possible to distribute the reagents simultaneously into the various channels. This arrangement has a particularly advantageous application in improving the throughput and reducing the number of distributions to be carried out.

[0011] The microfluidic substrate of the present invention is preferably semi-disposable (used for a few hundred reactions or some tens of hours) and is added on, in a removable fashion, to the thermal support, the fluid feed devices and the detection means. The control of the temperature, the movement of the fluids, the injection of the reagents, the mixing of the solutions in continuous flow and the detection are entirely automated. In addition, the combination of a permanent device and a disposable but relatively inexpensive microfluidic substrate allows a considerable reduction in costs relative to systems in which everything is integrated on the same microfluidic device.

[0012] One embodiment of the present invention is a device comprising a microfluidic substrate comprising at least one pathway for sample flow and at least one thermal transfer member which is capable of cycling between at least two temperatures, said at least one thermal transfer member being adapted to bring at least a portion of said sample pathway to said at least two temperatures while a sample is continuously flowing along said at least a portion of said sample pathway. In some aspects of this embodiment, the device further comprises a force supplying member operably linked to said at least one pathway for

sample flow wherein said force supplying member applies a force to said sample such that said sample travels along said at least one pathway. The device may further comprise a sample supplier which supplies a sample to said at least one pathway. The device may also further comprise at least one inlet basin positioned at a first end of said at least one pathway such that said sample supplier supplies said sample to said inlet basin and said sample travels from said inlet basin to said at least one pathway. The device of may also further comprise at least one outlet basin positioned at a second end of said pathway. In some aspects of the present invention, the device further comprises at least one reagent supplier positioned between said inlet basin and said outlet basin. In other aspects of the present invention, the device comprises a plurality of said pathways. The pathways may comprise channels arranged in parallel. The force generated by said force supplying member may be pressure. The microfluidic substrate may consist essentially of silicon. The device may further comprise a detector for measuring a physicochemical property of said biological sample. The thermal transfer member may comprise a metal bar in fluid communication with a plurality of water sources containing water at said at least two temperatures, said metal bar being in thermal communication with said at least a portion of said sample pathway.

[0013] Another embodiment of the present invention is a method for conducting a biochemical or chemical process comprising cycling at least a portion of at least one sample flow pathway between at least two temperatures while a sample comprising the reagents for said biochemical or chemical process is flowing through said at least a portion of said at least one sample flow pathway. The sample flow pathway may be located on a microfluidic substrate. The sample flow pathway may be in thermal communication with at least one thermal transfer member which cycles between said at least two temperatures while said sample is continuously flowing through said at least a portion of said at least one sample flow pathway. The thermal transfer member may cycle through said at least two temperatures a plurality of times while said sample is continuously flowing through said at least a portion of said at least one sample flow pathway. The thermal transfer member may cycle through said at least two temperatures from about 2 to about 35 times while said sample is continuously flowing through said at least a portion of said at least one sample flow pathway. In some aspects of this embodiment, at least a portion of a plurality of sample flow pathways are